

CLAIMS

WE CLAIM:

1. An isolated excisable polynucleotide comprising, a desired trait polynucleotide and a recombinase polynucleotide operably linked to a promoter, all flanked by a pair of directly oriented recombination sites, wherein the recombinase activity is regulatable.
2. The isolated polynucleotide of Claim 1, wherein the recombinase is selected from the group consisting of, ϕ C31, FLP, CRE, resolvase, SSV1-encoded integrase, R, Gin and transposase.
3. The isolated polynucleotide of Claim 1, wherein the recombinase is ϕ C31.
4. The isolated polynucleotide of Claim 3, wherein the ϕ C31 recombinase polynucleotide comprises an intron.
5. The isolated polynucleotide of Claim 3, wherein the ϕ C31 recombinase polynucleotide comprises a sequence as shown in SEQ ID NO:9 or SEQ ID NO:10.
6. The isolated polynucleotide of Claim 1, further comprising a selectable marker polynucleotide that is also flanked by the pair of recombination sites.
7. The isolated polynucleotide of 1, wherein the promoter is active in a plant cell, but inactive in a prokaryote.
8. The isolated polynucleotide of Claim 1, wherein the promoter is developmentally regulated.
9. The isolated polynucleotide of Claim 8, wherein the promoter is selected from the group consisting of a seed-preferred, leaf-preferred, root-preferred, pollen-preferred, egg-preferred promoter, germination-preferred, meristem-preferred, tuber-preferred, ovule-preferred and anther-preferred.
10. The isolated polynucleotide of Claim 8, wherein the promoter is a seed-preferred promoter.
11. The isolated polynucleotide of Claim 8, wherein the promoter is a germination-preferred promoter.
12. The isolated polynucleotide of Claim 8, wherein the promoter is a pollen-preferred promoter.
13. The isolated polynucleotide of Claim 1, wherein the promoter is environmentally regulated.

14. The isolated polynucleotide of Claim 13, wherein the promoter is regulated by an environmental factor or condition selected from the group consisting of, heat-shock, pathogen attack, anaerobic conditions, elevated temperature, decreased temperature, the presence of light and a chemical factor.
15. The isolated polynucleotide of Claim 13, wherein the promoter is a heat shock activated promoter.
16. The isolated polynucleotide of Claim 13, wherein the recombinase activity is repressible.
17. The isolated polynucleotide of Claim 16, wherein the promoter is repressed by a chemical.
18. The isolated polynucleotide of Claim 17, further comprising a chemically activated transactivator that represses the promoter, wherein in the transactivator is also flanked by the recombination sites.
19. The isolated polynucleotide of Claim 16, wherein the recombinase polynucleotide is operably linked to a chemical ligand receptor domain of a nuclear receptor.
20. The isolated polynucleotide of Claim 19, further comprising a chemically activated transactivator that represses the promoter, wherein in the transactivator is also flanked by the recombination sites.
21. A plant cell comprising the excisable polynucleotide of any of Claims 1-20. ✓
22. A plant comprising the plant cell of Claim 21.
23. The plant of Claim 22, wherein the plant is a dicot.
24. The plant of Claim 22, wherein the plant is a monocot.
25. A seed produced by the plant of Claim 22.
26. The seed of Claim 25, further comprising a chemical coating, wherein the chemical represses expression of the recombinase polynucleotide or represses the activity of a recombinase polypeptide encoded by the recombinase polynucleotide.
27. A tree comprising the excisable polynucleotide of Claim 1.
28. An isolated ϕ C31 recombinase polynucleotide comprising an intron.
29. The isolated ϕ C31 recombinase polynucleotide of Claim 28, wherein the polynucleotide comprises a sequence as shown in SEQ ID NO:9 or SEQ ID NO:10.
30. A method of producing a transgenic plant containing an isolated excisable polynucleotide comprising,

- a. introducing into a plant cell the isolated excisable polynucleotide, wherein the excisable polynucleotide comprises a desired trait polynucleotide and a recombinase polynucleotide operably linked to a promoter, all flanked by a pair of recombination sites in direct orientation, wherein the recombinase activity is regulatable; and
- b. generating from the plant cell the transgenic plant.
31. The method of Claim 30, wherein the recombinase is selected from the group consisting of, ϕ C31, FLP, CRE, resolvase, SSV1-encoded integrase, R, Gin and transposase.
32. The method of Claim 30, wherein the recombinase is ϕ C31.
33. The method of Claim 32, wherein the ϕ C31 recombinase polynucleotide comprises an intron.
34. The method of Claim 32, wherein the ϕ C31 recombinase polynucleotide comprises a sequence as shown in SEQ ID NO:9 or SEQ ID NO:10.
35. The method of Claim 30, wherein the excisable polynucleotide further comprises a selectable marker polynucleotide that is also flanked by the pair of recombination sites.
36. The method of Claim 30, wherein the promoter is active in a plant cell, but inactive in a prokaryote.
37. The method of Claim 30, wherein the promoter is developmentally regulated.
38. The method of Claim 37, wherein the promoter is selected from the group consisting of a seed-preferred, leaf-preferred, root-preferred, pollen-preferred, egg-preferred promoter, germination-preferred, meristem-preferred, tuber-preferred, ovule-preferred and anther-preferred.
39. The method of Claim 37, wherein the promoter is a seed-preferred promoter.
40. The method of Claim 37, wherein the promoter is a germination-preferred promoter.
41. The method of Claim 37, wherein the promoter is a pollen-preferred promoter.
42. The method of Claim 30, wherein the promoter is environmentally regulated.
43. The method of Claim 42, wherein the promoter is regulated by an environmental factor or condition selected from the group consisting of, heat-shock, pathogen attack, anaerobic conditions, elevated temperature, decreased temperature, the presence of light and a chemical factor.
44. The method of Claim 42, wherein the promoter is a heat shock activated promoter.

45. The isolated polynucleotide of Claim 42, wherein the recombinase activity is repressible.
46. The isolated polynucleotide of Claim 45, wherein the promoter is repressed by a chemical.
47. The isolated polynucleotide of Claim 46, further comprising a chemically activated transactivator that represses the promoter, wherein in the transactivator is also flanked by the recombination sites.
48. The isolated polynucleotide of Claim 45, wherein the recombinase polynucleotide is operably linked to a chemical ligand receptor domain of a nuclear receptor.
49. A method of expressing an excisable transgenic trait in a plant, comprising
- providing a plant comprising an excisable polynucleotide, wherein the excisable polynucleotide comprises a desired trait polynucleotide and a recombinase polynucleotide operably linked to a promoter, all flanked by a pair of recombination sites in direct orientation; and
 - exposing the plant to a condition or factor that represses activity of the recombinase.
50. The method of Claim 49, wherein the recombinase is selected from the group consisting of, ϕ C31, FLP, CRE, resolvase, SSV1-encoded integrase, R, Gin and transposase.
51. The method of Claim 49, wherein the recombinase is ϕ C31.
52. The method of Claim 51, wherein the ϕ C31 recombinase polynucleotide comprises an intron.
53. The method of Claim 51, wherein the ϕ C31 recombinase polynucleotide comprises a sequence as shown in SEQ ID NO:9 or SEQ ID NO:10.
54. The method of Claim 49, wherein the promoter is repressed by an environmental factor or condition selected from the group consisting of, a chemical, heat-shock, pathogen attack, anaerobic conditions, elevated temperature, decreased temperature and the presence of light.
55. The method of Claim 49, wherein factor is a chemical.
56. The method of Claim 55, wherein the excisable polynucleotide further comprises a chemically activated transactivator that represses the promoter, wherein in the transactivator is also flanked by the recombination sites.

57. The method of Claim 55, wherein the recombinase polynucleotide is operably linked to a chemical ligand receptor domain of a nuclear receptor.

58. A method of gene stacking in a cell comprising:

- a. introducing into the cell a first excisable polynucleotide comprising a first desired trait polynucleotide and a recombinase polynucleotide operably linked to a repressible promoter, all flanked by a first pair of recombination sites in direct orientation;
- b. introducing into the cell a second excisable polynucleotide comprising a second desired trait polynucleotide operably linked to a promoter, all flanked by a second pair of recombination sites in direct orientation, wherein the second pair of recombination sites are more efficiently excised than the first pair of recombination sites; and
- c. culturing the cell under a condition that represses activity of the recombinase.

59. The method of Claim 58, wherein the first recombination sites contain one or more point mutations.

60. The method of Claim 58, wherein the first recombination sites contain one or more deletions.